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HPTLC Method for Determination of Carbazole Alkaloid from *Murraya koenigii* Leaves

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Abstract:
A simple and rapid High Performance Liquid Chromatography (HPTLC) technique has been developed for the determination of bioactive marker compound carbazole alkaloid (Mahanimbine) in *Murraya koenigii* leaves. The petroleum ether (60-80ºC) extract of *Murraya koenigii* was subjected to column chromatography and eluted successively with petroleum ether and chloroform mixture. The fractions obtained were subjected to HPTLC analysis to identify the bioactive marker, carbazole alkaloid (Mahanimbine) in the collected fractions. HPTLC was performed with silica gel $60F_{254}$ plates with solvent system petroleum ether and chloroform (7:3 v/v) as mobile phase. Detection of bioactive marker compound (carbazole alkaloid) was performed by scanning the developed plate at 254nm. The results indicated that fractions obtained with 50% petroleum ether (60-80ºC) in chloroform showed single band of carbazole alkaloid (Mahanimbine). Therefore, HPTLC is simple and rapid technique and can be used to determine the bioactive marker compounds in the plant extracts.

Keywords: HPTLC, *Murraya koenigii*, Carbazole alkaloid

INTRODUCTION

Murraya koenigii (Rutaceae), commonly known as Curry patta is the one of the most well known plant. It is traditionally used as anti-emetic, anti-diarrhoeal, blood purifier and antioxidant activity [1, 2]. The *Murraya koenigii* leaves have been reported for anti-diabetic activity in diabetic rats [3]. In addition, the curry leaves has been reported for hypolipidemic activity in the albino rats [4].

The most important constituents of *Murraya koenigii* leaves are carbazole alkaloids (Mahanimbine, girinimbine, koenimbine and koenigicine). The carbazole alkaloids have been reported to have wider range of pharmacological properties such as antioxidant, anti-cancer and antibacterial activities [5, 6, 7].

Thus *Murraya koenigii* extract and its biological compounds such as carbazole alkaloids could be potential candidates for the treatment of various diseases.

HPLC method has been used for the determination of carbazole alkaloid [8]. However, High performance thin-layer chromatography (HPTLC) is a rapid, precise and cost-effective method and this method is widely used for the determination of biological compounds from medicinal plants.

Thus HPTLC method has been developed for the determination of the carbazole alkaloid (Mahanimbine, (Figure 1) in the *Murraya koenigii* leaves.

METHODS

PLANT MATERIALS

Murraya koenigii leaves (Rutaceae) were collected from the locality of IIT Kharagpur campus, West Bengal, India in the month of September and October 2007. The leaves were inspected to be healthy and botanically identified and authenticated by M. Senthilkumar, Plant Biotechnologist, Prathyusha Institute of Technology and Management, Chennai. The herbarium *Murraya koenigii* leaves was deposited in the Prathyusha Institute of Technology and Management (PITAM) against voucher no. PITAM/CH/00009/2007. *Murraya koenigii* leaves after collection were dried at room temperature (27-30ºC) for 25-30 days.

After complete drying, the dried materials were grounded into fine powder using a domestic electric grinder (Product: GX 21, Bajaj appliances, Mumbai, India) and used for extraction.

EXTRACTION AND ISOLATION

The dried plant powder of *Murraya koenigii* leaves were extracted with petroleum ether (60-80ºC) in a Soxhlet apparatus for 72 h. at room temperature. The total extract was concentrated under reduced pressure and kept at room temperature.

A greenish solid (3.6%) was separated out. This was dissolved in petroleum ether (60-80ºC) and chromatographed using silica gel (60-120 mesh) column and eluted successively with petroleum ether and chloroform mixture. Each collected fraction was tested for the presences of alkaloids by Dragendroff's test.

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY:

Chromatography was performed on silica gel $60F_{254}$ (10 cm× 10 cm; 0.25 mm layer thickness; Merck). Petroleum ether extract of *Murraya koenigii* (10mg/ml) and collected fractions residue (1mg/ml) was subjected to HPTLC (CAMAG, Switzerland) analysis. Extract and each fractions were spotted on a silica gel $60F_{254}$ (Merck, Darmstadt, Germany) TLC plate.

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The plate was air dried and then developed by using the solvent system petroleum ether and chloroform (7:3) in a CAMAG- twin-trough glass chamber previously saturated with mobile phase vapor for 20 min. After developing the plate, it was dried at 105ºC for 15 min and then it was scanned using Scanner 3 (CAMAG, Switzerland) at 254 nm using WinCATS 4 software.

RESULTS AND DISCUSSION

The bioactive marker compound (carbazole alkaloid) was determined by HPTLC technique. In column chromatography, the fraction obtained with 50% petroleum ether (60-80ºC) in chloroform showed positive test with Dragendroff's reagent. Figure 1 shows the HPTLC profiles of petroleum ether extract of *Murraya koenigii* and collected fractions from column chromatography.

In HPTLC technique, carbazole alkaloid (mahanimbine) was determined by using solvent system (petroleum ether and chloroform (7:3v/v) as mobile phase. The fractions obtained with 50% petroleum ether (60-80ºC) in chloroform showed a single band of carbazole alkaloid, which gave peak at R_f 0.34 for mahanimbine (Figure 2 & 3).

Thus this HPTLC technique can be considered as an accurate and precise method for the determination of carbazole alkaloid in *Murraya koenigii* samples.

CONCLUSION

The HPTLC technique expressed for the determination of carbazole alkaloid (Mahanimbine) from *Murraya koenigii* is simple, precise and can be used for the standardization of bioactive marker compounds in the plant extracts. This HPTLC technique is highly adaptable, because of the precision and repeatability of compound analysis in plant extracts. Therefore, HPTLC technique can be used for determination of bioactive marker compounds in other plant materials.

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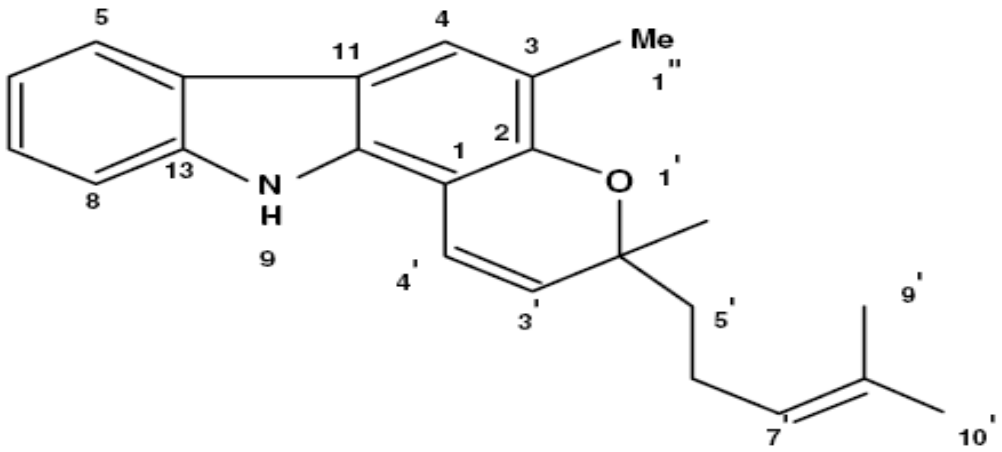

Figure 1: Structure of Mahanimbine (Carbazole alkaloid)

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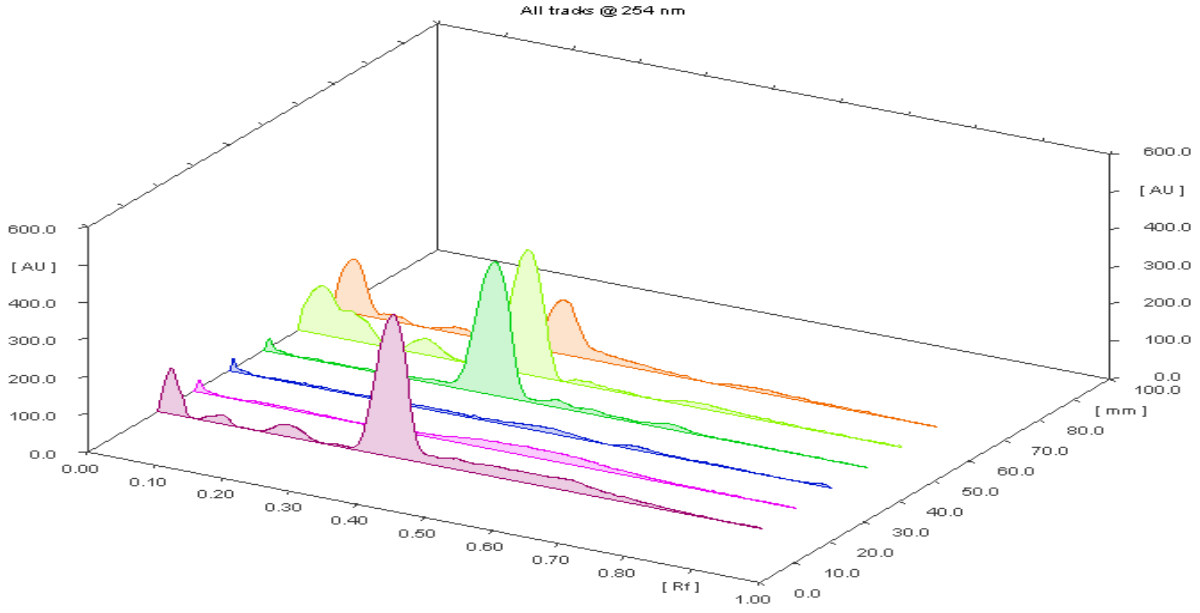

Figure 2: HPTLC profile of Colum fractions

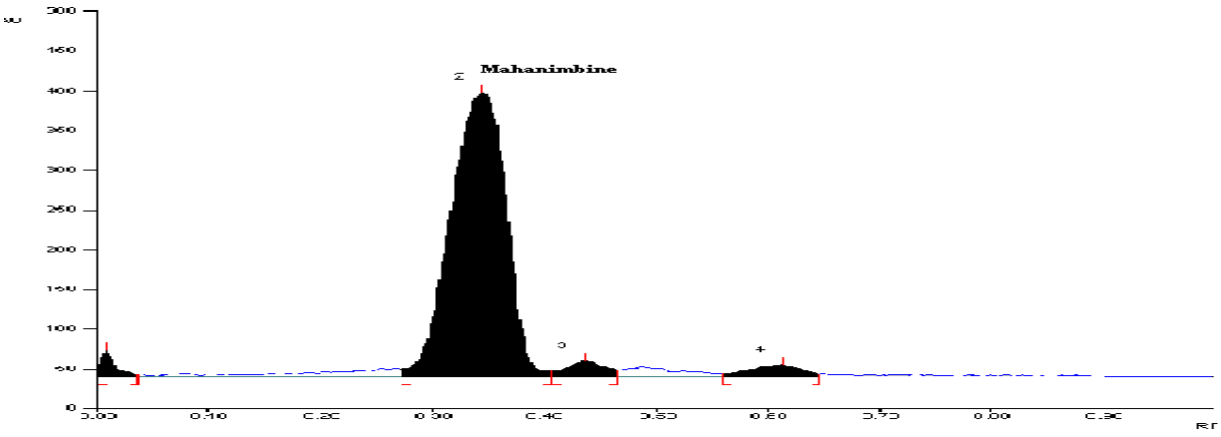

Figure 3: HPTLC profile of Mahanimbine (Carbazole alkaloid)